AMENDMENTS TO SPECIFICATION

In the Specification:

Please replace the paragraph beginning on page 6, line 21 and ending on page 7 line 2 with the following paragraph:

-- 1. Separation of ~~the~~ a sample from the vacuum allows direct visualization of wet samples. This immediately obviates the need for all dehydration procedures, including water replacement and critical-point drying. ~~The~~ A wet state most closely resembles the native state of the sample, preserving features that are distorted or destroyed during dehydration. This advantage is particularly important in the observation of tissues, where the true architecture involves both cells and extracellular matrix. In addition, the presence of fluid in and around the sample allows efficient dissipation of electrical charge and of excess heat. This eliminates artifacts due to sample charging, as well as thermal damage. --

Please replace the paragraph beginning on page 7, line 3 and ending on page 8 line 2 with the following paragraph:

-- 2. Electron microscopy of biological tissues is most frequently done in two imaging modes. Transmission electron microscopy (TEM) utilizes electrons transmitted through the sample; the entire thickness of the sample contributes to the image. Transmission techniques impose a severe constraint on the thickness of the samples: typically, 50 nm, which can be increased to 3 μm in ultrahigh voltage microscopes. Scanning electron microscopy uses a reflective mode, most frequently detecting secondary electrons that image only the surface topography of the sample. The non-vacuum SEM technique uses backscattered electron detection in a scanning electron microscope. The electron beam penetrates into the sample, and the backscattered electrons reveal sample features beyond the sample surface to a depth

of up to a few microns. Thus, although an electron scanning/reflecting mode of imaging is employed, the image is not limited to the surface, and internal structure of the sample is revealed. Furthermore, because detection is done in a reflective mode, any material lying beyond the interaction volume has no effect on imaging. Therefore, the samples can be of a thickness far exceeding the imaged region. Typically, a tissue fragment several millimeters thick can be viewed; only the material layer of a few micrometers or less that is closest to the surface contributes to the scanned image, without interference from the bulk of the sample. The thickness of the imaged region can be modulated by varying the acceleration voltage of the electron beam. Non-vacuum SEM thus yields "virtual sections" without the need for actual sectioning of the sample. This eliminates the need for embedding or freezing the sample, which ~~are~~ is otherwise required to enable sectioning of the sample. Finally, the dependence of electron backscattering efficiency on the material composition of the sample (through the atomic number Z) creates contrast even in the absence of heavy metal staining that is characteristic of TEM imaging. Subcellular organelles can be distinguished based on differences in local concentrations of lipids, phosphates, and salts within biological samples; and a wide variety of stains and labels can be used to enhance contrast. --

Please replace the paragraph beginning on page 17, line 15 with the following paragraph:

-- There is even further provided in accordance with still another preferred embodiment of the present invention [[A]] a method of characterizing chemical entities including applying a chemical entity to cells in a SEM-compatible sample enclosure and scanning the cells in a scanning electron microscope while in an environment characterized by a pressure exceeding the vapor pressure of water. --

Please replace the paragraph beginning on page 17, line 21 with the following paragraph:

-- Preferably, the method also includes analyzing changes in cell shape. Additionally or ~~laternatively~~ alternatively, the method also includes analyzing the cytoskeleton of the cells. Additionally, the method also includes analyzing the distribution of biomolecules in the cells. --

Please replace the paragraph beginning on page 29, line 1 with the following paragraph:

-- Figs. 96A and 96B and 96C are SEM micrographs, at ~~two~~ three different magnifications, of a fragment of murine heart prepared and imaged in accordance with a preferred embodiment of the present invention; --

Please replace the paragraph beginning on page 30, line 6 with the following paragraph:

-- Figs. 107A and 107B are SEM micrographs, at two different magnifications, of commercial 1.5% fat cow's milk, prepared and imaged in accordance with ~~stll~~ still another preferred embodiment of the present invention; --

Please replace the paragraph beginning on page 32, line 2 with the following paragraph:

-- The present invention relates to methods for electron microscopic inspections of wet biological and environmental samples at a non-vacuum environment. More specifically, the patent application relates to methods for visualizing samples in a scanning electron microscope (SEM) without the need for dehydration procedures including water replacement

and critical-point drying, which can destroy important structural detail and introduce artifacts in the sample to be observed. Absent the methods of the present invention, samples to be examined in an electron microscope must be held in a vacuum or a near vacuum to permit unimpeded access to the electron beam, which can only travel in a vacuum or near vacuum. --

Please replace the paragraph beginning on page 32, line 12 with the following paragraph:

-- The methods of the present invention advantageously employ a novel SEM sample container, described hereinbelow, into which the sample to be scanned is placed. The sample's hydration and atmospheric pressure state is maintained therein, even after placement on the SEM stage ~~and~~ when the SEM scanning chamber is evacuated. --

Please replace the paragraph beginning on page 34, line 16 with the following paragraph:

-- Reference is now made to Figs. 6A, 6B & 6C, which are three sectional illustrations showing the operative orientation of the SEM compatible sample container of Figs. 1A - 5B at three stages of operation. Fig. 6A shows the container of Figs. 1A - 5B containing a liquid sample 130 and arranged in the orientation shown in Fig. 1B, prior to threaded closure of enclosure elements 100 and 102. It is noted that the liquid sample does not flow out of the liquid sample enclosure 116 due to surface tension. The electron beam permeable, fluid impermeable, membrane 110 is seen in Fig. 6A to be generally planar. --

Please replace the paragraph beginning on page 36, line 20 with the following paragraph:

-- Fig. 8B illustrates closing of the container containing the sample 160. Fig. 8C shows the closed container, in the orientation of Fig. 1B, being inserted onto [[a]] the stage 144 of [[a]] the SEM 146. It is appreciated that there exist SEMs wherein the orientation of the container is opposite to that shown in Fig. 8C. --

Please replace the paragraph beginning on page 39, line 22 and ending on page 40, line 2 with the following paragraph:

-- Reference is now made to Figs. 16A, 16B & 16C, which are three sectional illustrations showing the operative orientation of the SEM compatible sample container of Figs. 11A - 15B at three stages of operation. Fig. 16A shows the container of Figs. 11A - 15B containing a liquid sample 230 and arranged in the orientation shown in Fig. 11B, prior to threaded closure of enclosure elements 200 and 202. It is noted that the liquid sample does not flow out of the liquid sample enclosure 216 due to surface tension. The electron beam permeable, fluid impermeable, membrane 210 is seen in Fig. 16A to be generally planar. --

Please replace the paragraph beginning on page 42, line 7 with the following paragraph:

-- Fig. 18B illustrates closing of the container containing the sample 260. Fig. 18C shows the closed container, in the orientation of Fig. 11B, being inserted onto [[a]] the stage 244 of [[a]] the SEM 246. It is appreciated that there exist SEMs wherein the orientation of the container is opposite to that shown in Fig. 18C. --

Please replace the paragraph beginning on page 51, line 15 with the following paragraph:

-- Connecting element 1122 preferably has a recess 1124. Connecting element 1122 is also formed with a protrusion 1126, seen in Figs. 35A & ~~35B,protruding~~ 35B, protruding into recess 1124. --

Please replace the paragraph beginning on page 53, line 18 with the following paragraph:

-- Reference is now made to Fig. 37, which is a simplified sectional and pictorial illustration of the tissue containing sample and insertion into a SEM using the SEM compatible sample container of Figs. 31A - 36C. --

Please replace the paragraph beginning on page 54, line 9 with the following paragraph:

-- Fig. 38B shows the container of Fig. 38A immediately following full threaded engagement between enclosure elements 1100 and 1102 and connecting element 1122 producing sealing of ~~the~~ a cell sample enclosure, here designated by reference numeral 1178, from the ambient. It is noted that the sample containing cells 1172 is in close contact with the electron beam permeable, fluid impermeable, membrane 1110 due to the force exerted by the positioner 1176. It is seen that the electron beam permeable, fluid impermeable, membrane 1110 and its supporting grid 1112 bow outwardly due to pressure buildup in the cell sample enclosure 1178 as the result of sealing thereof in this manner. --

Please replace the paragraph beginning on page 58, line 24 and ending on page 59, line 2 with the following paragraph:

-- Reference is now made to Figs. 46A, 46B & 46C, which are three sectional illustrations showing the operative orientation of the SEM compatible sample container of

Figs. 41A-45B at three stages of operation. Fig. 46A shows the container of Figs. 41A-45B containing a tissue sample 1260 and arranged in the orientation shown in Fig. 41B, prior to threaded closure of enclosure elements 1200 and 1202 and connecting element 1222. The electron beam permeable, fluid impermeable, membrane 1210 is seen in Fig. 46A to be generally planar. --

Please replace the paragraph beginning on page 64, line 7 with the following paragraph:

-- Preferably, sealing cover 1454 is provided with an array of positioners 1480. Individual positioners 1480 are suspended within coils 1482, as shown in ~~Fig.51C~~ Fig. 51C, so as to move non-liquid samples up and against electron beam permeable, fluid impermeable, membrane 1210 (shown in Figs. 41A-50) seated in sample dish 1475. --

Please replace the paragraph beginning on page 65, line 1 with the following paragraph:

-- Preferably, sealing cover 1504 is provided with an array of positioners 1520, shown in Fig. ~~53B.Individual~~ 53B. Individual positioners 1520 are suspended within coils 1522, as shown in Fig. 53B, so as to move non-liquid samples up and against electron beam permeable, fluid impermeable, membrane 1510. --

Please replace the paragraph beginning on page 72, line 1 with the following paragraph:

-- Fig. 65B illustrates closing of the container containing the sample 2160. Fig. 65C shows the closed container, in the orientation of Fig. 58B, being inserted onto stage 2144

of SEM 2146. It is appreciated that there exist SEMs wherein the orientation of the container is opposite to that shown in ~~Fig.65C~~ Fig. 65C. --

Please replace the paragraph beginning on page 75, line 23 and ending on page 76, line 5 with the following paragraph:

-- The diaphragm 2218 is inserted into a first aperture 2222 formed in an exterior surface of a wall of the second enclosure element 2202. A second aperture 2223, shown in ~~Fig.~~ Figs. 72A&72B, is formed in an interior surface of a wall of the second enclosure element 2202. First aperture 2222 and second aperture 2223 enable diaphragm 2218 to provide pressure relief by defining a fluid communication channel between one side of the diaphragm 2218 and the environment in which the SEM compatible sample container is located. A plug 2224 is preferably provided to retain the diaphragm 2218 in aperture 2222. Plug 2224 is preferably formed of a ring 2225 having a generally central aperture 2226 and is attached to an internal surface of second enclosure element 2202 defined by aperture 2222 by any conventional means, such as by a tight fitting engagement. --

Please replace the paragraph beginning on page 77, line 12 with the following paragraph:

-- Fig. 73B shows the container of Fig. 73A immediately following full threaded engagement between enclosure elements 2200 and 2202, producing sealing of the liquid sample enclosure 2216 from the ambient. The light guide tip 2238 is shown to be immersed in liquid sample 2239. It is seen that the diaphragm 2218 bows outwardly due to pressure buildup in the liquid sample enclosure 2216 as the result of sealing thereof in this manner. In this embodiment, electron beam permeable, fluid impermeable, membrane 2210 and its supporting grid 2212 also bow outwardly due to pressure buildup in the liquid sample enclosure 2216 as the result of sealing thereof in this manner, however to a significantly lesser extent than in the ~~embodiement~~ embodiment of Fig. 63B, due to the action of diaphragm 2218. This can be seen by comparing Fig. 73B with Fig. 63B. --

Please replace the paragraph beginning on page 90, line 3 with the following paragraph:

-- Electron gun assembly 3010 also preferably includes a safety valve system comprising an airlock 3040 and a top wall 3042 defining a safety valve system internal volume 3044. Prior to removal of container 3000 the airlock 3040 is locked, so as to maintain an evacuated environment within internal volume 3044, typically at a vacuum of 10^{-2} - 10^{-6} millibars, during container removal. After the airlock 3040 is locked, a gas, typically nitrogen, is introduced via an inlet tube 3046 into internal volume 3032 of electron gun assembly 3010. Container 3000 is then removed and preferably replaced. Alternatively, another container 3000 may then be placed in ~~sample dish~~ electron gun assembly 3010. After container 3000 or another container is introduced into the electron gun assembly 3010, the gas is pumped out, typically through an outlet tube 3048, by a pump (not shown) and airlock 3040 is unlocked. --

Please replace the paragraph beginning on page 91, line 4 with the following paragraph:

-- Fig. 86B shows a plurality of slabs 3050, including a top slab 3066, providing a tissue sample thickness of X1. The slicing instrument 3060 slices the tissue sample 3064 into two portions, top portion 3068 and bottom portion ~~3070~~ 3069, where resulting bottom portion ~~3070~~ 3069 has a thickness of X1. --

Please replace the paragraph beginning on page 92, line 17 with the following paragraph:

-- In accordance with another preferred embodiment of the present invention, cells are maintained under cell culture conditions, such as immersion in growth medium, for example, LB medium at 37°C for bacteria or in Dulbecco's modified essential medium (DMEM) supplemented with 10% fetal bovine serum, at 37°C in a humidified atmosphere including 5% CO_2, for cultured animal cells, in a SEM compatible sample container, or in a subassembly thereof, prior to imaging. In this embodiment, the SEM compatible sample container or subassembly serves as an experimental vessel, analogous to conventional vessels, such as ~~petri~~ Petri dishes, cell culture dishes, cell culture flasks, and / or multiwell plates, for growth and/or manipulation of cells. Additionally, samples including cells and other samples may be subjected to various treatments, including, but not limited to, addition of mitogens, drugs, hormones, cytokines, antibiotics, toxic materials, viruses, bacteria, vital stains or other staining solutions, mixing (co-culture) of different cell types, transfection of cells with DNA, irradiation with ultraviolet, X-ray or gamma radiation, replacement of the growth medium with other media, such as media lacking serum, while in the sample container or subassembly, in accordance with the objectives of an experiment or an analysis. --

Please replace the paragraph beginning on page 96, line 7 with the following paragraph:

-- In this embodiment, the method includes additional steps to provide close contact with the partition membrane. To maintain suitable contact with the partition

membrane, a sample is provided with a fairly smooth surface. This surface may be the "natural" edge of the sample, such as an epithelial lining that is part of a tissue sample, or may be generated by cutting a tissue piece with a razor or a slicing instrument, preferably by the device and methods depicted in Figs. 86A - 87B, or in another preferred embodiment, with a mechanized instrument, such as a vibratome®. Additionally, in this embodiment, the tissue typically needs to be pushed against the partition membrane, such as by a positioning device, preferably, the device shown in Figs. 31A - 50. --

Please replace the paragraph beginning on page 103, line 11 with the following paragraph:

-- The Myelin sheath of nerve processes are prominent lipid-rich structures, which are clearly visible in the methods of the present invention. Changes in the extent of nerve myelination and in the structure of the nerve fibers and the associated myelin sheaths accompany a wide variety of clinical neurodegenerative situations, such as autoimmune diseases such as Multiple Sclerosis, Guillain-Barré Syndrome, congenital storage diseases such as Neuronal Ceroid Lipofuscinoses (OMIM 256730, possibly the most common group of neurodegenerative diseases in children), complications of infectious diseases such as ~~diphteria~~ diphtheria, HIV, or prion diseases; and trauma. In these cases, the methods of the present invention can contribute to diagnosis based on rapid, high resolution imaging of small samples, such as biopsies or cerebrospinal fluid, to histopathological analysis in either patients or in animals. Additionally, the ability to observe the organization of neural tissue at high resolution can be employed in the analysis of suspected tumors in the nervous system. --

Please replace the paragraph beginning on page 103, line 26 and ending on page 104, line 13 with the following paragraph:

-- The extracellular matrix (ECM), which is composed mostly of protein, glycoproteins, oligosaccharide and polysaccharide chains, is the structural foundation of tissues and organs. The ECM plays crucial roles in diverse processes, including

morphogenesis and organogenesis, regulation of cell growth, migration and polar extension, such as axonal growth. Several genetic and degenerative diseases are associated with alterations in the structure of the extracellular matrix, such as scurvy due to vitamin C deficiency, which leads to incomplete modification of collagen and general dissolution of connective tissues; collagen modification in aging, resulting in changes in connective tissue; genetic diseases due to mutations in collagen genes, such as Osteogenesis imperfecta (Collagen I mutations), The Helers-Danlos ~~Syntrome~~ Syndrome, and various arthritic conditions. Additionally, cancer cells may have specialized interactions with the surrounding extracellular matrix, for example, cancer cells produce and secrete enzymes that change the structure of the surrounding matrix. Such production and secretion of enzymes may be associated with specific properties of the cancer cells, including metastatic activity, which may have important implications for prognosis and treatment. --

Please replace the paragraph beginning on page 104, line 24 and ending on page 105, line 6 with the following paragraph:

-- The methods of the present invention provide a direct and effective means of viewing epithelia. An epithelial cell layer is placed directly in a sample container so that it is in contact or in close proximity to a partition membrane, the container is sealed and placed in a SEM, and imaged by scanning electron microscopy according to an embodiment of the present invention. The epithelial layer may be imaged while attached to an underlying tissue, or may be removed from such tissues before imaging. ~~Additionally,the~~ Additionally, the epithelial layer may occur without such attachment in its natural position in the body prior to removal for imaging. Applications include the diagnosis of many diseases that affect the structure of epithelia, such as diseases of the digestive system, the respiratory system, endocrine and exocrine glands, vascular diseases, skin diseases, eye diseases. --

Please replace the paragraph beginning on page 112, line 22 and ending on page 113, line 16 with the following paragraph:

-- Biomolecules such as proteins often function as dimers or larger oligomers, in which the subunits may be identical (homodimers, homo-oligomers) or different (heterodimers and hetero-oligomers). For example, the EGF receptor, also designated HER1, can form dimers with an other HER1 molecule, or with any of a group of similar receptor molecules termed HER2, HER3 and HER4; in fact, the HER1 – 4 proteins can associate in a variety of homodimers and heterodimers. The formation of each such dimer may be differentially influenced by binding of any of several EGF-like ligands, with different biological consequences. Finally, the HER family of receptors are differentially expressed in some types of cancer; a much-discussed example is the high prevalence of HER-2 in some breast cancers. Indeed, high prevalence of HER2 in a cancer is liked to a poor prognosis, and drugs such as Herceptin® target this receptor specifically. The association of receptors and other proteins into dimers and larger oligomers has been investigated using bulk techniques, in which (for example) a general crosslinking molecule is used to covalently link molecules that are in close proximity, or by distance-dependent energy transfer between ~~fluerent~~ fluorescent molecules and ~~orophores~~ chromophores attached to different proteins. The methods of the present invention uniquely allow to measure the extent of association of similar or different biomolecules, by using labeling each of the biomolecules with a label that can be distinguished from each other by SEM inspection according to the methods of the present invention. Non-limiting examples of such distinguishable labels are colloidal gold particles of different sizes (e.g. 10 nm and 20 nm, 15 nm, 25 nm and 35 nm, etc.), and combination of electron-dense labels with cathodoluminescence labels, visualized in the same sample by simultaneous detection of backscattered electrons and light. --

Please replace the paragraph beginning on page 115, line 8 with the following paragraph:

-- Reference is now made to Fig. 88, which depicts schematically the main protocol steps that comprise the method of the present invention. It is appreciated that not all ~~embodiemnts~~ embodiments will comprise all of the steps enumerated hereinbelow. It is

appreciated that the methods herein disclosed provide for a desirable degree of flexibility in preparing and visualizing the sample. --

Please replace the paragraph beginning on page 125, line 7 with the following paragraph:

-- Peform specific ~~labelling~~ labeling procedure as described above. If subsequent visualization reveals problems with excessive background signal, add a mild detergent such as Triton X-100/NP40 (1% v/v) to the wash buffer used after incubation with primary antibody. --

Please replace the paragraph beginning on page 134, line 24 and ending on page 135, line 4 with the following paragraph:

-- As known in the art, the transfection procedure results in uptake and expression of the transfected DNA in a fraction of the cell population, whereas other cells do not take up or express any of the transfected gene. This is clearly seen in Fig. 92, where, for example, one cell 3230 whose nucleus 3232 is clearly visible as a bright oval, is heavily stained with the gold-linked antibody, as seen in ~~the~~ bright patches 3234, whereas a neighboring cell is not stained by the antibody, its nucleus 3236 being visible due to its inherent material contrast from the cytoplasm. This image exemplifies the excellent signal to noise characteristics achievable with the methods of the present invention. --

Please replace the paragraph beginning on page 137, line 21 and ending in page 138, line 4 with the following paragraph:

-- Reference is now made to Figs. 96A and 96B and 96C, which are SEM micrographs of a fragment of murine heart inserted without treatment into a sample container and imaged in a wet state in accordance with a preferred embodiment of the present invention, preferably according to the description in Figs. 41A – 50, at ~~two~~ three different magnifications. Note the general arrangement of cells 3270 seen in at lower magnification in Figs. 96A - 96B, and the intracellular details (nucleus 3272 and the bright organelles 3274, probably mitochondria) seen at higher magnification in Fig. 96C. Membrane supporting grid, shown for example Figs. 96A - 96C demonstrate again the ability provided by methods of the present invention to generate imaging contrast from natural material distribution in the sample, but more importantly, the ability to generate an image in a very short time (5-10 minutes) after obtaining the tissue, which may be a biopsy or a sample taken during surgery. --

Please replace the paragraph beginning on page 143, line 24 and ending on page 144, line 10 with the following paragraph:

-- Reference is now made to Figs. 110A and 110B, which are micrographs of CHO cells obtained by backscattered electron detection and light detection, respectively, in a scanning electron microscope, in accordance with a preferred embodiment of the present invention, preferably according to Figs. 58A – 77. The cells are grown on the partition membrane 2110 and imaged directly without treatments such as fixation or staining. Fig. 110A shows an image generally similar to that seen in Fig. 89, whereby the general outline 3440 of a cell, a bright region 3442 indicating a nucleus, and dark spots 3444 indicating lipid droplets. The contrast in this image is due to material differences reflected in efficiency of electron backscattering. Fig. 110B is an image obtained concurrently, derived from photons emitted from the sample during SEM scanning. In this image, the outline 3450 of the cell, ~~the~~ a bright region 3452 indicating the nucleus, and bright spots 3454 indicating lipid droplets. The contrast in Fig. 110B is derived from totally distinct mechanisms than the contrast in Fig. 110A, namely from efficiency of cathodoluminescence. In this case, the

cathodoluminescence image may yield unique information on material distribution within the specimen. --

Please replace the paragraph beginning on page 144, line 20 with the following paragraph:

-- Reference is now made to Figs. 112A - 112B, which depict SEM inspection of samples using X-ray detection in accordance to a preferred embodiment of the present invention. Fig. 112A shows a SEM inspection of an aqueous sample of HeLa cells in water in a sample container such as shown in Figs. 11A – 20, using X-ray spectroscopy. The analysis identifies oxygen 3474 as the major component, with carbon 3472 at a lesser amount; these are the expected results from analysis of cells in water. ~~Fog.~~ Fig. 112B shows a similar analysis of a vacuum grease; here carbon 3476, oxygen 3477 and ~~flurine~~ fluorine 3478 are the predominant elements. --